1 Biogeochemical characteristics of settling particulate organic matter in
2 Lake Superior: a seasonal comparison

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4 Hongyu Li ^{a,1*}, Elizabeth C. Minor ^b

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6 ^a *Large Lakes Observatory and Water Resources Science Program, University of Minnesota,*
7 *Duluth, MN 55812, USA*

8 ^b *Large Lakes Observatory and Department of Chemistry and Biochemistry, University of*
9 *Minnesota Duluth, Duluth, MN 55812, USA*

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11

12

13

14 ¹Current address:

15 Earth and Ocean Sciences, 701 Sumter Street, EWS 617, University of South Carolina,
16 Columbia, SC 29208, USA.

17 Phone: +0012182697516

18 Email: hli@geol.sc.edu

19 *Corresponding author

20

ABSTRACT

To assess settling particulate organic matter (POM) seasonality and its availability to the benthic community, settling particulate matter was studied in terms of mass fluxes and main biogeochemical characteristics (including organic carbon (OC), nitrogen, and stable carbon and nitrogen isotopic values) at two Lake Superior offshore sites over the course of a year. Fourier transform infrared spectroscopy (FTIR) and hydrolysis, extraction, and derivatization were used to provide further compositional information. Carbon and nitrogen content, isotopic and wet chemical data, and FTIR spectra show that summer particulate material is mainly autochthonous, with higher proportions of amide and carbohydrate. FTIR shows that spring particulate material contains relatively high proportions of clay minerals, indicating major sources from sediment resuspension and/or spring runoff. Distinct amino acid distributions at the two sites, revealed by principal component analysis (PCA) based on amino acid mol% composition, possibly result from differences in OM sources and the degree of degradation occurring at the two sites. Carbohydrate (PCHO), total hydrolyzable amino acid (THAA) and FTIR data suggest that the nutritional value of bulk POM to benthic heterotrophs should be lower in spring than summer-fall, although both periods exhibited high sinking fluxes of total mass and OC. Due to sediment resuspension events and an oxic water column, organic matter eventually buried in Lake Superior's sediments has probably experienced extensive alteration due to several cycles through the water column and the bacterially-active sediment-water

interface.

1. Introduction

Particulate organic matter (POM) in aquatic ecosystems plays significant roles in the global carbon cycle by serving as a food source for heterotrophic organisms and by transporting organic matter produced in the euphotic zone to the sediments. During its transport, POM undergoes biogeochemical changes such as uptake by biota, remineralization and exchange with dissolved organic matter (DOM), so only a portion of the surface production eventually reaches the surface sediments (Wakeham et al., 1997; Hedges et al., 2000; Burdige, 2007). POM, by its innate composition and its reactivity, affects the cycles of global carbon, nutrients, and trace metals as well as anthropogenic pollutants (Eppeley and Peterson, 1979; Sholkovitz and Copland, 1981; Baker and Eisenreich, 1989; Baker et al., 1991). Knowledge of the POM molecular level composition, fluxes and contribution to total particulate fluxes as well as its variations is necessary not only for investigating the source and dynamics of POM but also for a better understanding of degradation and selective preservation of the macromolecules in POM. For benthic ecosystems, which are mainly fueled by settling POM, different components of the POM pool are of different nutritive values. For example, just in terms of energy equivalents, average particulate protein has been estimated to contain 24.0 J/mg as compared to an average particulate carbohydrate value of 17.5 J/mg and an average lipid value of 39.5 J/mg (Gnaiger, 1983; Navarro

and Thompson, 1995). The relative contributions of energetically variable, bioavailable and nutrient-containing organic matter can fluctuate seasonally (Pusceddu et al., 1996). Studies of temporal and spatial changes in the biochemical composition of sinking particles can therefore help us assess the nutritional quality of this material, which impacts the dynamics of benthic communities. Although detailed characterization of POM is important, it is difficult because POM is heterogeneous, consisting of living and dead cells, fecal pellets, aggregates, organically-coated mineral grains and other materials.

Carbohydrate and protein are among the major biochemicals produced by photosynthetic processes in the euphotic zone and are generally considered bioavailable for aquatic organisms. They represent a significant component of the particulate organic matter in the water column (Lee and Cronin, 1982, 1984; Pakulski and Benner, 1992) and in sedimentary material. Particulate carbohydrates (PCHOs) comprise 5–20% of the total particulate organic carbon (POC) in marine sediments (Cowie and Hedges, 1992; Burdige et al., 2000). Amino acids, the building blocks of protein molecules, comprise from < 1% to as much as 67% of the organic carbon (OC), and 10–100% of the organic nitrogen in open-ocean net plankton, water column POM and sediments (Lee and Cronin, 1984; Lee, 1988; Wakeham et al., 1997). The distribution of carbohydrates and amino acids in POM mainly depends on the source of the organic matter, the depositional rate, the water depth and local hydrological factors. It primarily reflects the balance between biological production and biological

consumption through the journey of those compounds to the bottom of the ocean or lake. Understanding the temporal and spatial changes in quantity and quality of particulate organic matter, including its carbohydrate and amino acid components, is important to the study of benthic heterotrophic activities by microbes and macroorganisms. Wet chemical extraction and detection methods have been developed and applied as classical ways to quantify carbohydrates and amino acids in marine and freshwater POM samples (Johnson and Sieburth, 1977; Lindroth and Mopper, 1979).

Different techniques have been applied to heterogeneous POM for the identification and characterization of its chemical components such as flow cytometry in conjunction with fluorochromes (Moreira-Turcq and Martin, 1998; Kerner et al., 2003), flow cytometry in conjunction with pyrolysis mass spectrometry (Minor et al., 1998; Minor and Eglinton, 1999), nuclear magnetic resonance (NMR) spectroscopy (Hedges et al., 2000; Lee et al. 2004), and Fourier transform infrared (FTIR) spectrometry (Bruns et al., 2010; Tremblay et al., 2011). Among these, FTIR is inexpensive, non-labor intensive and can quickly provide an overview of compound class or functional group composition in complex mixtures of organic matter based upon analysis of a small amount (~1 mg) of raw dried sample (Abdulla et al., 2010). FTIR analysis is based on the fact that vibrations of different covalent bonds absorb infrared radiation at different wavenumbers resulting in an IR spectrum that can be used for structural characterization. However, as happens with DOM samples, the

heterogeneity of POM samples acts to simplify their FTIR spectra, which exhibit broad bands resulting from overlap of multiple functional groups (Li et al., 2013). This feature is complicated further by the fact that POM is often intimately associated with clays and other inorganic components that also contain covalent bonds adsorbing in the infrared range. Thus, this study applies classical wet extraction and derivative molecular techniques for the analysis of carbohydrates and amino acids, and stable isotope analyses, along with FTIR characterization of sinking POM. Our main aims were to investigate the origin and biochemical composition of settling POM in the open-water region of Lake Superior over seasonal scales and to estimate POM's nutritional value for benthic consumers.

2. Material and methods

2.1. Study site and sampling details

Lake Superior is the Earth's largest lake by surface area (82,100 km²), with a maximum depth of over 400 m, mean depth of 150 m, water residence time of 180 years, and is dimictic, mixing twice annually, once in late spring-early summer (usually April to June) and once in late fall-early winter (December to January) (Herdendorf, 1982; Urban et al., 2005; Austin, 2008). Largely affected by the mixing events, sediment resuspension occurs several times a year in the lake during the unstratified periods and estimates of its contribution to settling fluxes of OC range from 10–30% in offshore sites (Urban et al., 2004). In general, Lake Superior has

biogeochemical similarities to many open ocean systems such as low water column primary production ($\sim 200\text{--}350\text{ mgC/m}^2/\text{d}$, Sterner et al., 2004), low dissolved OC ($89\text{--}208\text{ }\mu\text{M}$) and particulate organic carbon (POC) ($2.3\text{--}16.5\text{ }\mu\text{M}$) concentrations (Zigah et al., 2012), small terrestrial impact, and the dominance of a microbial food web (Cotner et al., 2004). Thus the study of Lake Superior and comparisons with both lakes and oceanic systems can provide valuable information for a broad audience. The OC deposition in Lake Superior sediments is about $0.48 \times 10^{12}\text{ gC/yr}$ (McManus et al., 2003) to $1.5 \times 10^{12}\text{ gC/yr}$ (Johnson et al., 1982; Klump et al., 1989). Surface sediment at open lake sites has OC contents ranging $1.5\text{--}4.3\%$ (Li et al., 2013). Dominant signals from clay minerals/biogenic silica along with carbohydrate, carboxylic acid, aliphatic/acetyl ester, amide/protein and phenol/lignin have been identified using FTIR in the Lake Superior surface sediments (Li et al., 2013).

To obtain settling POM samples in the hypolimnion, McLane Parflux 21 cup sequential sediment traps with a diameter of 0.92 m^2 were deployed with moorings located in the eastern (EM, 150 m, total water depth 240 m) and western (WM, 150 m, total water depth 180 m) arms of the lake, as shown in Fig. 1, from October 2009 to June 2010. (Note: our site EM, which is the same as in Zigah et al. (2012), is considerably farther east, and actually within the eastern basin of the lake, as compared to the sampling location EM in Sterner et al. (2004). On the same moorings, temperature and pressure loggers were deployed at a variety of depths from the surface to $150\text{--}200\text{ m}$. The upper part of the WM mooring failed in December 2009,

and the remainder was recovered and the mooring rebuilt in June 2010 at 125 m depth to continue to collect summer samples. The deep sediment trap sampling collecting portion of the WM mooring remained intact through winter 2009/2010 as indicated by mooring design (location of floats, sensors, and the trap) and pressure sensor data. The EM trap was recovered and reset in June 2010 at 125 m depth to collect samples until September 2010. Physical data presented here for onset of mixing and stratification are from the EM mooring; for onset of summer stratification, site EM generally lags site WM by one to two weeks (J. Austin, personal communication). For both WM and EM, sample cups were programmed to rotate and collect descending particulate matter for 10/11 days during winter and 3/4 days during summer over an entire year. Because splits of these samples were used for DNA and RNA analyses on a separate project, only deionized water was added to each cup and no preservative was used. Material captured in the sediment traps was then equally split into 10 fractions with a McLane sediment trap splitter. Fractions of the captured material were freeze dried and homogenized prior to further analysis. For summer trap samples, every 3 or 4 continuous trap samples, which represent settling POM of 10 or 11 days in total, were mixed and measured as one sample. The middle day of the entire 10-11 days is used in the discussion and figures that follow to represent each collection of sample.

2.2. *Elemental and isotopic analyses*

Acid fumigation (Harris et al., 2001) was used to remove carbonate carbon before elemental analysis (EA) and isotope ratio mass spectrometry (IR–MS). Weighed aliquots of sample were placed in clean Ag capsules and 50 μ L MilliQ water was added to each aliquot. The samples were then fumed over 12 M HCl (6–8 h), oven dried (60 °C, 4 h) and cooled. Each sample (in the Ag capsule) was enclosed within a Sn capsule before analyzing for TOC, TN, stable carbon isotope ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) ratios. One subset of samples was analyzed with a Costech CNS analyzer interfaced with a ThermoFinnigan Delta PlusXP stable isotope ratio mass spectrometer at the Large Lakes Observatory. The typical standard deviations for the instrumentation based on analyses of multiple external standards were 0.2‰ for $\delta^{13}\text{C}$ and 0.17‰ for $\delta^{15}\text{N}$. A second subset of samples was analyzed with a Micro Cube elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer at the University of California Davis Stable Isotope Facility. The long-term standard deviation for this instrumentation was reported to be 0.2‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$. The stable isotope ratios are reported using delta notation (δ). The final delta values are expressed in permil (‰) relative to international standards V-PDB (Vienna PeeDee Belemnite) and air for carbon and nitrogen. The C/N ratio reported was calculated as the molar ratio of TOC to TN of each sample.

2.3. FTIR measurement

Spectra were obtained with a Nicolet Magna IR 560 FTIR ESP spectrometer equipped with purge gas generator unit by collecting 200 scans from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1} and using Happ-Genzel apodization (Bretzlaff and Bahder, 1986). For expelling CO_2 contamination from the air, 1 lag time (5 min) was employed between closing the analytical chamber and starting the analysis. Freeze dried, homogenized (but not acid treated) samples were run as KBr pellets (1 mg sample, 150 mg KBr, homogenized in a mortar and pestle, 1500 psi). Each pellet was dried in a desiccator (> 6 h) before analysis. A pure KBr pellet (KBr dried for 2 h at 105 °C prior to use and then placed in a desiccator overnight) was analyzed before each sample analysis as a background blank. Spectra were converted to absorbance units and baseline corrected. The second derivatives of these spectra were generated using Origin 7.0 and the second order Savitzky-Golay smoothing with 11 convolution points (Savitzky and Golay, 1964; Helm and Naumann, 1995).

2.4. Total carbohydrates analysis

The MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride) method of carbohydrate analysis (Johnson and Sieberth, 1977) was applied to quantify total carbohydrates. The procedures applied in this study are adapted from Pakulski and Benner (1992) and described briefly here. A known amount of each sample was first hydrolyzed with 12 M (72 wt%) H_2SO_4 for 2 h, diluted to 1.2 M and then further

hydrolyzed for 3 h at 100 °C. The diluted hydrolysate was neutralized and then reduced with freshly prepared ice cold 10% KBH₄ to form formaldehyde. Then replicates of 2 mL aliquots of the resulting hydrolysate were oxidized with periodic acid. After reaction with MBTH and yellow-green color development, absorbance was recorded immediately at 635 nm with a spectrophotometer equipped with a 1 cm quartz cuvette. All chemicals used were ACS grade or $\geq 98\%$ purity. The amount of carbohydrate present in the samples was calculated by comparing the absorbance of the unknown samples with the absorbance of glucose standards. Then the amount of glucose equivalent carbon in the sample was estimated based on carbon being 40% by weight in glucose. PCHO-C/TOC was used to represent the carbohydrate carbon normalized to TOC in each sample.

2.5. Total amino acid analysis: derivatization

Precolumn o-phthalaldehyde (OPA)/mercaptopropionic acid (MPA) derivatization combined with HPLC was employed to determine THAA (adapted from Lindroth and Mopper, 1979; Mengerink et al., 2002; Frank and Powers, 2007), which are reported as mass of the summed amino acids relative to dry sample weight (g). The mass of THAA carbon normalized to TOC (THAA-C/TOC) and the mass of THAA nitrogen normalized to TN (THAA-N/TN) are also reported. Ten samples, chosen to cover multiple seasons and both sites, were hydrolyzed with 3 mL of 6 N HCl (ACS grade) at 110 °C for 19 h (Lee and Cronin, 1982) prior to derivatization and HPLC analysis.

The OPA/MPA derivatization reagent was prepared fresh each day by mixing, in order of listing, 6.25 mL methanolic OPA (2 mg/mL in HPLC grade methanol), 10.0 mL borate buffer (0.2 M; pH = 9.9) and 25 μ L MPA solution. The final pH of the derivatization reagent was 9.36 ± 0.05 . Each dried hydrolysate sample was dissolved in 500 μ L of MilliQ water and filtered through a Whatman Puradisc 4 mm Syringe Filter (0.2 μ m, PES membrane, polypropylene housing). The filters were not rinsed prior to filtration of the samples; however, a method blank was collected and measured together with samples. Aliquots (5 μ L) of the filtered hydrolysate were then mixed with 500 μ L of the borate buffer (0.2 M; pH = 9.9) and derivatized with 40 μ L of OPA/MPA reagent immediately before HPLC analysis.

The Shimadzu HPLC system consisted of a LC-10A7 dual piston pump, a RF-10AXL fluorescence detector and a SIL-10A autosampler, all controlled by the SCL10A system controller for chromatographic analysis. PeakSimple 3.29 software was used for data collection. The separation of the amino acid derivatives was achieved on a Waters (Milford, MA) XBridge Shield RP18 column (100 mm x 4.6 mm i.d., 3.5 μ m particle size). The column temperature was 30 $^{\circ}$ C. The flow rate of the mobile phase was 1 mL/min. Detection was performed fluorometrically with an excitation wavelength of 340 nm and an emission wavelength of 455 nm. Gradient elution involving two mobile phases was employed. Mobile phase A consisted of 0.02 M sodium acetate buffer mixed with 0.3% THF adjusted to pH 7.2 with 6M NaOH. Mobile phase B consisted of HPLC grade water and acetonitrile in a 1:1 ratio. All

250 buffers were filtered through a 0.2 μm filter and degassed with ultrapure helium. The
251 total HPLC run time for the separation of the derivatized amino acids in a single
252 sample or standard was 40 minutes. The gradient timetable is given in Appendix 1.
253 HPLC grade methanol, acetonitrile and the Pierce Amino Acid Standard H were
254 obtained from Fisher Scientific. Deionized water was processed through a Milli-Q
255 purification system (Millipore, USA). All solid phase chemicals were ACS degree or >
256 98% purity.

258 **3. Results**

259 *3.1. Mass fluxes, OC fluxes and stable carbon isotope analysis*

260 Settling particulate mass fluxes and OC fluxes determined by sediment traps from
261 the east basin (EM, 150 m) and west basin (WM, 125 to 150 m) of Lake Superior are
262 shown in Fig. 2. The settling particulate mass fluxes ranged from 4.4–382 $\text{mg}/\text{m}^2/\text{d}$ at
263 EM and 60–592 $\text{mg}/\text{m}^2/\text{d}$ at WM. These fluxes are consistent with previous settling
264 mass fluxes (1–1200 $\text{mg}/\text{m}^2/\text{d}$) measured from 2005 to 2008 at 145 m depth at WM of
265 Lake Superior (Woltering et al., 2012). These fluxes were considerably lower than
266 earlier values (560–1940 $\text{mg}/\text{m}^2/\text{d}$) measured at the same depth in Lake Michigan
267 (Eadie et al., 1984; Meyers and Eadie, 1993). Our Lake Superior mass fluxes (Fig. 2a)
268 were much more variable than mass fluxes at 500 m depth in the open ocean (at the
269 Bermuda Atlantic Time Series station BATS from 1984 to 1998; Conte et al., 2001).
270 Lake Superior's minimum fluxes (at 125–150 m water depth) were similar to the

minimum fluxes at BATS (at 500 m water depth), but its maximum fluxes were approximately 6 times higher than the highest recorded at 500 m at BATS from 1984 to 1998 (Conte et al., 2001). Lake Superior's peaks in mass flux occurred in late December/early January and May (Fig. 2a) at both WM and EM. The timing of maximum mass flux is consistent with data in Woltering et al. (2012).

OC fluxes ranged from 0.6–23.1 mgC/m²/d at EM and 3.7–30.8 mgC/m²/d at WM. These values are comparable to carbon flux measurements by Heinen and McManus (2004) from 1999 to 2000 at 250 m water depth (~35 meters above the bottom) at a different site in the western arm of Lake Superior; they found fluxes varied from 6.0–43.2 mgC/m²/d with a minimum during winter and a maximum during spring. However, these values, like the total mass flux values, are much lower than in other Laurentian Great Lakes (Eadie et al., 1984; Meyers and Eadie, 1993). The Lake Superior values are also somewhat lower, on a yearly average basis, to POC fluxes at 150 m depth in the open ocean (at the Bermuda Atlantic Time Series station, BATS) with Lake Superior's maximum POC fluxes (at 125–150 m), roughly equivalent to the median fluxes the same depth at BATS (Buesseler et al., 2007). The maximum POC fluxes at both EM and WM, like the peaks in total mass flux, occurred in late December/early January and May (Fig. 2a,b).

Carbon and nitrogen contents in sinking material in the hypolimnion of Lake Superior are higher during summer stratification and lower during unstratified conditions, as seen in Fig. 3. WM contains higher carbon and nitrogen contents than

EM in the summer season and increases in the carbon and nitrogen contents occur after the onset of stratification. The EM and WM sites have similar seasonal variations in terms of molar C/N ratios and stable carbon and nitrogen isotope values. Except for one sample, the C/N values (10.0 ± 0.7 at EM and 9.8 ± 1.2 at WM) of all spring and winter samples vary little, with slightly lower C/N ratios in summer. The one exception is that the samples collected around Nov 26, 2009 at both EM and WM exhibit high C and N contents, a higher C/N ratio and more depleted $\delta^{13}\text{C}$ values. OC stable isotope ratios change little with seasons at both locations, being -27.9 to -29.7‰ at EM and -27.5 to -29.2‰ at WM.

3.2. Carbohydrate and amino acid distributions

PCHO-C/TOC ranges from 7.0–18.8% at EM and from 4.0–19.7% at WM. These are comparable to PCHO-C/TOC values in the Equatorial Pacific at depths of 105 m (18%) to 4000 m (5%) (Wakeham et al., 1997). PCHO-C/TOC increased at both sites from winter-spring to summer. However, WM and EM (Fig. 4a) showed very different spatial and temporal patterns in terms of the total PCHO-C flux. WM showed a steady increase from winter-spring to summer, while a pattern resembling POC mass fluxes (Fig. 3) was seen at EM site. PCHO-C (mg/g dry sediment weight) has the same variation as PCHO-TOC at both sites.

For the ten selected samples covering both sites and different seasons, as shown in Fig. 5, THAA content varies from 12.9–50.4 mg/g; THAA flux ranges from 0.3–9.7

313 $\text{mg/m}^2/\text{d}$; THAA–C/TOC ranges from –8.5 to 25.0% and THAA–N/TN ranges from
 314 31.3–90.2%. THAA contents, THAA–C/TOC and THAA–N/TN values at WM and
 315 EM (Fig. 5) showed different temporal patterns. WM showed a steady increase from
 316 winter-spring to summer, while a pattern similar to the POC mass fluxes was seen at
 317 EM site. The generally higher THAA contents in summer POM samples at both sites
 318 is consistent with their higher carbon and nitrogen contents. Although we do not have
 319 data for all samples, the THAA flux appears similar to the total mass flux profile (Fig.
 320 2) for that site. The THAA and THAA–C/TOC values were very similar to those seen
 321 in trap samples from ~1357 m water depth in the Equatorial Pacific (Gupta and
 322 Kawahata, 2000). THAA–N/TN values were about two-fold higher and THAA fluxes
 323 ranged from similar to 4-fold higher in Lake Superior than in Equatorial Pacific trap
 324 samples at ~1000 m depth (Wakeham et al., 1997; Gupta and Kawahata, 2000).
 325 Although total amounts of THAA in Lake Superior samples varied by a factor of 2–3,
 326 settling POM samples appeared to be rather similar in terms of the distributions of the
 327 individual amino acids (Table 1). In general, glycine/threonine, aspartic acid, histidine
 328 and alanine are the dominant species with the average contribution of 18.5, 13.5, 12.7
 329 and 10.1 mol%, respectively. However, the ratios of the four groups of amino acids
 330 vary among samples. Glutamic acid, serine, valine, cysteine, leucine, arginine and
 331 lysine hydrochloride/proline accounted for 3–10 mol%, and a minor group of
 332 isoleucine, phenylalanine, tyrosine and methionine contributed < 3 mol%. This
 333 distribution is very similar to the THAA distribution reported for coastal marine

sediment samples in Dauwe and Middelburg (1998).

Despite the overall similarity between samples, some molar composition differences can be seen (Table 1). Due to the overlapping of peaks in our analytical protocol (e.g., threonine and glycine, valine and cysteine), the Amino Acid based Degradation Index (Dauwe and Middelburg, 1998; further revised in Dauwe, 1999) is not applicable to our data. However, principal component analysis (PCA) based on the mol% composition of the 17 amino acids in the selected 10 sediment trap samples was applied in the same way as in Dauwe (1999) to investigate the variation of amino acid distribution among samples, as shown in Fig. 6. Principal component 1 (PC1), which accounts for 75.3% of the variability, is mainly driven by the difference between summer samples at EM and the winter WM sample. Winter samples for both sites are shifted into more positive PC1 space relative to their summer counterparts. Factor coefficients (principal component loadings) give the relation between the principle components and the original variables (mole percentage of protein amino acids). PC1 has negative coefficients for threonine/glycine, arginine and tyrosine and positive coefficients for all other amino acids; these differences drive the clustering of samples along PC1. Positive correlation can be found between PC1 score and THAA contents for samples from EM ($n = 5$, $R^2 = 0.7864$, $P = 0.0449$), with high THAA content summer samples having more negative PC1 values. However, no correlation was found between PC1 score and the THAA contents of samples from WM ($n = 5$, $R^2 = 0.1801$, $P = 0.4752$).

3.3. Compositional variation of POM as seen by FTIR

3.3.1. Infrared absorbance of POM and its second derivative spectra

FTIR spectroscopy was used to characterize (at the chemical functional group level) settling particulate material collected with sediment traps in Lake Superior. Fig. 7 shows FTIR absorbance spectra of individual samples collected at different times of the year at both EM and WM sites. As reported in previous FTIR studies of heterogeneous natural samples (DOM, lake sediments and clays) (Madejová and Komadel, 2001; Abdulla et al., 2010; Li et al., 2013), a relatively high degree of similarity among samples was observed. This is, in part, due to the overlap of peak responses of multiple functional groups, which broadens FTIR bands and simplifies the FTIR spectra. However, peak shifts and peak intensity differences can be seen as a function of sampling time, indicating seasonal changes in POM composition. To resolve overlap bands within each spectrum and to identify exact frequencies of peak responses, the second derivatives of the FTIR spectra were used (Smith, 1996; Griffiths and De Haseth, 2007). Below we have identified the main FTIR bands in spectra of all samples based on the peaks resolved by the second derivative spectra (e.g. Fig. 8).

All spectra show strong signals from clay minerals. The Si–O stretching at 1010 cm^{-1} and 1035 cm^{-1} , the Al–OH bending absorptions at 913 cm^{-1} , combining with the potential Si–OH stretching at 3620 cm^{-1} and 3698 cm^{-1} , indicate the presence of clay

minerals composed of Al and SiO₂, possibly kaolinite (Madejová, 2003), which are commonly present in Lake Superior (Bailey and Tyler, 1960; Weaver, 1989) and deep ocean sediments (Biscaye, 1964). According to Rosen et al. (2010) and Swann and Patwardhan (2011), the signal at approximately 1000 cm⁻¹ appears to be other silicate/clay minerals while the one near 1100 cm⁻¹ is most likely from biogenic silica, while the stretching vibrations of silanol Si–OH from biogenic silica (Smith, 1999) could also contribute to the bands appearing at 3698 and 3620 cm⁻¹. Quartz is indicated by signals appearing at ~800, 779, 695, 537 cm⁻¹ (Silverstein, 1996; Smith, 1999).

All spectra show the presence of OC as indicated by absorptions in the range 2800–3000 cm⁻¹. The broad O–H/N–H absorbance peak centered between 3280 and 3420 cm⁻¹, is attributed to the overlap of O–H stretching in organic alcohols, carbohydrate and carboxylic acid compounds or the N–H band of amide or even OH groups bonded to silicates or silica (Pandurangi et al., 1990). The peaks centered at 1000–1100 cm⁻¹ most likely result from Si–O stretching of clay minerals and biogenic silica in settling particulate materials. The multiple strong peaks within the 1200–1000 cm⁻¹ range (for instance, 1005, 1037 and 1097 cm⁻¹ in Fig. 8a) can also include C–O bands from carbohydrates. Carbohydrates occur in natural organic matter as many different forms of polysaccharides, monosaccharides, oligosaccharides or even conjugations of saccharides with proteins or lipids as glycol-conjugate compounds (Yang et al., 1995; Brandenburg and Seydel, 2002).

The compounds containing carbonyl C=O functional groups, including the carboxylic acids and esters, contribute to the small carbonyl absorbance peak centered between 1683 and 1748 cm^{-1} . Combining the carbonyl C=O signal with the C–C=O stretching around 1165–1185 cm^{-1} , the presence of aliphatic esters in POM can be confirmed. In addition to aliphatic esters, there is a signal consistent with the presence of the acetyl ester functional group, a possible C–C=O stretching band at $\sim 1238 \text{ cm}^{-1}$ (Celi et al., 1997; Smith, 1999), but this peak could also be contributed by C–O asymmetric stretching in phenol (Celi et al., 1997) and from C–O vibrations in carbohydrates. The large change in dipole moment during the C=O bond stretch gives FTIR the ability to detect small concentrations of amide functional groups (CON–), which are the primary building elements of protein and peptides in our samples. The peaks centered around 1651 cm^{-1} (Amide I, Silverstein, 1996; Smith, 1999) are believed to be Amide I while the peaks centered at 1540 cm^{-1} could be attributed to Amide II (Silverstein, 1996; Widjanarko et al., 2011), although bands in the 1530–1650 cm^{-1} region can also be attributed by alkene and aromatic unsaturation and water deformational modes (Silverstein, 1996).

The vibrations of lignin's aromatic rings, which show up as multiple peaks around 1515 cm^{-1} (Mascarenhas et al., 2000; Raiskila et al., 2007; Derkacheva and Sukhov, 2008) are observed in most POM samples throughout the year at both sites. However, the $\sim 1515 \text{ cm}^{-1}$ bands are present as very small contributions to the spectra (Fig. 8a, 1518 cm^{-1}). As these bands are fairly weak even in measurements of lignin isolates,

the intensity of these bands cannot be applied quantitatively.

Thus, throughout the year settling particulate matter from both sites exhibits strong signals from clay minerals (and some biogenic silica) and recognizable signals from the organic chemical functional groups. Functional groups of carbohydrates, carboxylic acids, aliphatic/acetyl esters, amides/proteins and phenols/lignins can be identified.

3.3.2. Seasonal variations of major functional groups of settling POM based on PCA and FTIR

To investigate the variation among samples at different times of the year at each site, PCA (Kvalheim et al., 1985; Sanni et al., 2002) was performed on the data sets composed of normalized FTIR spectra. The data matrices were z-scored and variance scaled before the PCA analysis was performed. Principal components 1 and 2 (PC1 and PC2) account for 76.7% of the variability in the EM data set. PC1 and PC2 account for 75.1% of the variability in the WM data set. As shown in Fig. 9, at both EM and WM sites, the winter-spring (Oct-May) samples tend to be clustered in negative PC1 space while in summer, the samples shift toward more positive PC1 values. A separation of samples from different seasons can be seen, with a tight clustering among winter-spring samples and a more scattered distribution of summer samples in the PCA plot. This PCA distribution of Lake Superior particulate samples based on FTIR shows that August to September samples drive the PCA 1 variation at

both sites.

Differences in the infrared absorbance (normalized to total sample absorbance) at characteristic frequencies can be used to compare the relative contributions of corresponding functional groups within a group of samples. Fig. 10 shows the FTIR-based seasonal variations in the contributions of the primary functional groups within the settling particulate material at both sites based on signals at 1651 cm⁻¹ (Amide I C=O stretching), 1540 cm⁻¹ (Amide II C=O stretching), 3410 cm⁻¹ (O–H stretching of carboxylic acid and carbohydrate), 1736 cm⁻¹ (aliphatic ester C=O stretching), 1097 cm⁻¹ (carbohydrate/biogenic silica/silicate minerals) and 1005 cm⁻¹ (carbohydrate/biogenic silica/silicate minerals). A similar seasonal pattern for Amide I, Amide II, the O–H stretch of carboxylic acid and carbohydrate and the C=O stretch of aliphatic ester was observed, with small fluctuations in terms of the contribution of these functional groups to the total particulate matter in the winter-spring season, and a significant increase in summer. The proportion of amides, carboxylic acid, carbohydrate and aliphatic ester within particulate matter increased after the onset of summer stratification. Positive correlation can be found between the Amide I peak normalized intensity and THAA contents at both EM (n = 5, R² = 0.8037, P = 0.0394) and WM (n = 5, R² = 0.7825, P = 0.0462) sites, with samples with higher THAA content having higher FTIR normalized Amide I peak intensity (data not shown). However, a weaker correlation (N = 10, R² = 0.4310, P = 0.0392) was found when data from the two sites were combined. Similar relationships are seen with Amide II.

EM samples were found to have slightly higher relative abundances of the Amide I, Amide II, carboxylic acid and aliphatic ester functional groups relative to WM samples.

A different seasonal pattern was seen for peaks at 1097 and 1005 cm^{-1} , the first of which could represent overlap of signals from carbohydrate and biogenic silica while the second is most likely an overlap of carbohydrate and silicate minerals (clay). The infrared absorbance of summer samples at these two wavelengths generally decreases relative to winter-spring. Considering that PCHO–C % increases in summer at both sites, the intensities at 1097 and 1005 cm^{-1} are therefore dominated by signals from biogenic silica and silicate minerals. According to Rosen et al. (2010) and Swan and Patwardhan (2011), the peak at 1097 cm^{-1} should be biogenic silica while the one at 1005 cm^{-1} is more likely to be other silicates. Biogenic silica increased during winter stratification and the first part of summer stratification, in contrast, other silicates show proportional increases during the mixed periods and decreases during stratification.

4. Discussion

4.1. POC origin, degradation and chemical characterizes in Lake Superior

The seasonal variations of particulate sinking fluxes, carbon, nitrogen and stable isotope composition appear to be associated with sediment resuspension and primary production. The two increases in settling flux at both WM and EM sites correspond to

the fall and spring mixing events. Water column mixings likely cause enhanced benthic fluxes from local sediment resuspension and downslope sediment transport in turbidity currents. The lower OC content of settling solids during unstratified conditions, as seen in Fig. 3, indicates a larger contribution from eroded clays and resuspended sediments, which have lower TOC contents (Li et al., 2013), to the settling flux at that time. The fact that OC fluxes have trends similar to the bulk particulate settling mass fluxes suggests that the particles originating from sediment resuspension may contribute both organic and mineral fractions to the settling POM pool. The higher carbon and nitrogen contents during summer stratification than in other seasons (Fig. 3a,b) indicate a larger proportional input by primary productivity in the summer and early fall. The slightly lower C/N ratios in summer are also consistent with higher inputs from primary production.

Settling POM at both sites has similar and relatively low molar C/N ratios suggesting that the dominant POC source is algal or microbial, as opposed to land-derived vascular plant material, which generally has C/N ratios greater than 15 (Meyers, 1994). The $\delta^{13}\text{C}$ values are similar to those obtained from the plankton tows in Lake Superior (-27 to -29‰), also suggesting that the settling POM is mainly derived from autochthonous productivity (Meyers and Ishiwatari, 1993; Ostrom et al., 1998). The one exception to this trend of primarily autochthonous POM is the samples collected around Nov 26, 2009 at both EM and WM. The more terrigenous signatures of these samples are most likely due to strong wind gusts and a rain event

(Weather underground archives, 2015) that happened in this time period causing a stronger influence from terrestrial inputs.

Although the two sites have similar seasonal variations in terms of OC, nitrogen and isotope contents, settling particulate material at WM contains higher carbon and nitrogen contents than at EM in the summer season. Increases in the carbon and nitrogen contents at both sites occur after the onset of stratification. During summer stratification, WM has both mass fluxes and POC mass fluxes approximately 4-fold higher than at EM (Fig. 2). The summer trends in composition and flux are likely due to stronger primary production at WM relative to EM, based upon chlorophyll concentrations and nutrient levels (Barbiero and Tuchman, 2004; Sterner, 2011).

Lake Superior is large enough, and has a sufficiently small watershed, to exhibit little direct terrestrial impact (in terms of freshwater inflows and land-derived materials) at open lake sites such as our study locations. Atmospheric input is another source of material to the lake, but this is thought to contribute very little OC compared to primary production (Urban et al., 2005). Based on modeled yearly water column primary production, 200–350 mgC/m²/d (Sterner, 2010), extensive degradation of primary production has occurred in the Lake Superior water column; only a small fraction of this primary production (approximately 0.2–11%) was recorded in our deep water sediment traps. Our data indicate even stronger loss terms than previously published by Baker et al. (1991) who estimated that 75% of OC settling from surface waters degrades during transit to the Lake Superior floor. POC loss in the Lake

Superior water column is similar to that seen in the open ocean, where water column degradation of organic matter removes more than 90% of the surface production before it reaches the sediments (e.g., Wakeham et al., 1997). Compared to the surface sediments (1.5–4.3% TOC; 0.2–0.5% TN; Li et al., 2013), the settling solids in our mid-hypolimnion traps are two to four fold more enriched in OC and organic nitrogen than in the sediments 30 to 115 m below, suggesting intensive degradation in the deep lake (most likely occurring at the sediment-water interface). Such degradation is consistent with differences seen between our POC fluxes and reported annual average sediment POC burial rate (0.25 mol/m²/year, equivalent to 8.2 mg/m²/d, Hales et al., 2008). The slightly higher C/N ratios and more enriched $\delta^{13}\text{C}$ values in the sediments (10.0–10.5 for C/N, –26.5 to –26.2‰ for $\delta^{13}\text{C}$; Li et al., 2013) relative to the settling POM also suggest a greater proportion of diagenetically altered OM in the sediments.

4.2. Seasonality of POM composition and nutritional quality

In addition to bulk parameters such as mass flux and %OC changes, there are also temporal changes in the composition of the OC being delivered, which potentially affect lower water column and benthic heterotrophic activities by microbes, zooplankton and macroorganisms. Based on FTIR, throughout the year settling particulate matter from both sites exhibits strong signals of clay minerals (and some biogenic silica) and recognizable signals from the organic chemical functional groups including carbohydrates, carboxylic acids, aliphatic/acetyl esters, amides/proteins and

phenols/lignins. PCA based on FTIR of both EM and WM samples reveals separation of samples from different seasons confirming compositional differences of the particulate material as a function of seasonality. The high compositional similarity among the winter-spring samples revealed by PCA is likely due to remineralization processes leading to a more similar residual particulate matter. In contrast, the more variable composition of summer samples compared to winter-spring samples is likely due to the contribution of labile POC in summer from the multiple and varied phytoplankton/microbial communities developing in the upper water column.

The seasonal variations in the contributions of the primary functional groups within the settling particulate material at both sites were assessed based on signals of their representative wavelengths. The increase in the proportion of amides, carboxylic acid, carbohydrate and aliphatic ester within particulate matter after onset of summer stratification is consistent with significantly elevated lake productivity in summer (Sterner, 2010) contributing a variety of labile compounds to the sinking POM pool. The proportional increase in biogenic silica during winter stratification and the first part of summer stratification is consistent with winter diatom production, as seen in Lake Erie (Twiss et al., 2012) and hypothesized to occur in Lake Superior, and summer production of diatoms (Fig. 10e). The elevation of the biogenic silica signal also coincides with the slight increases in amide 1 absorbance (Fig. 10a) and total POC flux (Fig. 2b) during winter stratification, which may be indicative of stratification and algal production related to ice formation in Lake Superior. The

silicates indicated by 1005 cm^{-1} , in contrast, show proportional increases during the mixed periods, and decreases during stratification, consistent with the idea of water column mixing leading to resuspension of silicate minerals from the sediments. The strong decreases in silicate mineral signals seen in summer-fall were likely due to the combination of a dilution effect from primary production and the reduced impact of sediment resuspension in that season. This understanding of the temporal and spatial changes in the quantity and quality of biogeochemically important functional groups can inform the study of benthic heterotrophic activities by microbes and macroorganisms.

Besides assisting in FTIR peak assignments, the measurement of PCHO-C (mg/g dry sediment), PCHO-C/TOC, THAA/TOC, and THAA (mg/g dry sediment) also help with the assessment of POM nutritional qualities and its temporal variation. Both PCHO-C and THAA increased at both sites from winter-spring to summer, suggesting that higher primary productivity enhanced transportation of both carbohydrates and amino acids (relative to inorganic material) to the lake bottom in the warmer, more productive summer season. In addition to the enhancement of these organic matter components relative to bulk sediment, the increase in PCHO/TOC (Fig. 4c) and THAA/TOC (Fig 5c) shows that the organic matter itself has changed in quality or biochemical composition; the increase in the proportion of OC consisting of THAA and PCHO indicates that this POM is probably a higher quality food source for the benthos. WM and EM exhibit different temporal patterns in terms of both PCHO-C

flux and THAA flux, possibly due to different degrees of import from primary production versus sediment resuspension at each site. The increase from winter-spring to summer at WM for PCHO–C/TOC, PCHO–C flux, THAA flux and THAA is in response to increasing primary production, while the PCHO–C flux (Fig 4b) and THAA flux (Fig 5b) patterns resembling POC mass fluxes at EM site suggests a stronger influence on both PCHO–C and amino acids by sediment resuspension. The mismatch of THAA to the May total POM mass flux peak is likely due to depleted THAA contents in the resuspended sediment material at this period of time.

PCA based on amino acid compositions of the selected samples suggests site-specific seasonal impacts on amino acid signatures possibly resulting from different settling POM sources (perhaps a different primary producer community structure) and a different extent of degradation at the two sites. Summer samples appear to be enriched with a different set of amino acids as compared to their winter counterparts at both sites and the degree of degradation drives the clustering of samples along PC1. The positive correlation between PC1 score and THAA contents for samples from EM but not WM, suggests that amino acid degradation is the main factor affecting amino acid distribution at EM. It is likely that other factors such as stronger primary production input or dilution effects from sedimentary material inputs are affecting the amino acid composition at the WM site. Thus, the amino acids in winter settling POM could be significantly different than summer samples in terms of bioavailability to benthic consumers.

Employment of the sum of carbohydrate, protein and lipid contents (weight percent of this sum relative to total particle mass) has been reported (Navarro and Thompson, 1995) as a good estimate of the edible fraction of particles delivered to the benthos. We have extended this idea to estimating the food quality of the particulate organic matter by calculating the C contribution to total POC from this potentially more biolabile organic C fraction. While lacking quantitative lipid data (lipid concentrations are generally much smaller than carbohydrate and amino acid concentrations), such estimates were performed by comparing the sum of PCHO–C/TOC (Fig. 4) and THAA–C/TOC (Fig. 5) for winter-spring vs summer values in this study. At both Lake Superior sites, the organic content of sinking particles was estimated to be 2 to 3 fold higher in nutritional value to the benthic consumer in summer than in winter-spring.

5. Conclusions

Isotopic analysis, C and N contents, carbohydrate and amino acid analyses, and FTIR coupled with PCA were used to characterize settling particulate material and to study the spatial and temporal patterns of particulate matter sedimentation in Lake Superior. The origin and biochemical composition of sinking particles were investigated to reveal total particle and POM nutritional value to benthic consumers.

Similar seasonal patterns were observed at both sites with the highest mass flux occurring in the unstratified season; however, the most organic-rich samples were

obtained from the summer stratified season. Processes controlling the type and quantity of sinking solids in the water column of the Lake Superior appear to be both resuspension of surficial sediments and primary production.

Increases in PCHO–C/TOC values, THAA contents, THAA–C/TOC and THAA–N/TN were seen at both sites corresponding to enhanced primary productivity input in summer. However, distinct amino acid distributions as revealed by PCA did not appear related to primary production trends, but possibly resulted from differences in OM sources and the degree of degradation occurring at the two sites. The combination of PCHO–C/TOC and THAA–C/TOC suggests that summer POM has 2 to 3 fold higher nutritional values to the benthic consumer than spring-winter POM in Lake Superior.

Based on FTIR spectra, clay minerals and biogenic silica are dominant in all samples obtained through the year. Despite the presence of a fully oxygenated water column, which usually facilitates major degradation of organic matter, the settling fluxes contained a measurable fraction of organic matter that included functional groups representative of carbohydrate, carboxylic acid, aliphatic/acetyl ester, amide/protein and phenol/lignin. Principal component analysis of FTIR data from the sediment trap material suggests compositional variation as a function of season, with relatively strong inorganic clay mineral signals dominating in winter-spring and a strong signal from amide, carbohydrate and other organic components in summer. Due to both the % organic matter present and the composition of that organic matter,

the relative bioavailability and nutritional values of sinking particles to benthic microbes should be lower in winter than summer, although both the onset of winter and late-spring to early summer exhibited peaks in total mass and POC flux.

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Figure Captions

Fig. 1. Lake Superior. Stars indicate sampling sites.

Fig. 2. Seasonality of (a) total particulate mass fluxes and (b) POC mass fluxes in the fall 2009- spring 2010 period in Lake Superior. Three or four continuous summer trap samples (after Jun 17, 2010) were averaged to obtain the same resolution as winter trap samples (10 or 11 days). The symbols and sample dates indicate the midpoint of sample cup deployment periods. For Figures 2–5 and 10, black dashed lines indicate onset of fall mixing (Dec 10, 2009), winter stratification (Jan 31, 2010), spring mixing (Apr 2, 2010), and summer stratification (Jun 10, 2010) based upon thermistor data from the EM mooring.

Fig. 3. Seasonality of (a) carbon contents in total settling material; (b) stable carbon isotope values; (c) nitrogen contents in total settling material; (d) stable nitrogen isotope values; (e) molar ratio of carbon vs nitrogen. Three to four continuous summer trap samples, which represent settling POM of 3–4 days, were mixed and measured as one sample to match the sampling durations at other seasons. The symbols and sample dates indicate the midpoint of sample cup deployment periods. As in Fig. 2, black dashed lines indicate fall mixing, winter stratification, spring mixing, and summer stratification.

Fig. 4. Seasonal variation of (a) PCHO–C in mg/g dry weight; (b) PCHO–C flux; c) carbohydrate carbon percent in total POC in the fall 2009 to spring 2010 in Lake Superior. As in Fig. 2, black dashed lines indicate onsets of fall mixing, winter stratification, spring mixing, and summer stratification.

Fig. 5. Seasonal variation of (a) THAA in mg/g dry weight; (b) THAA flux in the fall 2009 to spring 2010 period in Lake Superior; (c) THAA–C/TOC; (d) and THAA–N/TN based on the five selected samples at EM and WM. As in Fig. 2, black dashed lines indicate onsets of fall mixing, winter stratification, spring mixing, and summer stratification.

Fig. 6. PCA projection based on mol% composition of the 17 THAAs in the selected 10 sediment trap samples. EM samples are shown with filled black squares and WM samples are shown with filled dark gray stars. The data matrixes were z-scored and variance scaled before the PCA analysis was performed.

Fig. 7. 1D FTIR spectra of all settling particulate matter samples collected at different times of the year at (a) EM site, (b) WM site. The colors indicate different samples. Each spectrum was normalized by total area before comparison to reduce variation from sample loading/processing.

Fig. 8. The FTIR spectrum and its second derivative for sinking particulate samples from (a) winter (Jan. 10, 2010 to Jan 21, 2010) and (b) summer (Aug 10, 2010 to Aug 15, 2010) at WM.

Fig. 9. PCA projection of normalized FTIR data for (a) EM and (b) WM.

Fig. 10. Seasonality of POM compound class distributions in Lake Superior at EM (gray) and WM (black) sites based on absorbance intensity (normalized to total sample absorbance) at wavelengths of (a) 1651 cm^{-1} representing Amide I C=O stretching, (b) 1540 cm^{-1} representing Amide II C=O stretching, (c) 3410 cm^{-1} representing O–H stretching of carboxylic acid and carbohydrate, (d) 1736 cm^{-1} representing aliphatic ester C=O stretching, (e) 1097 cm^{-1} representing overlap of carbohydrate, biogenic silica/silicate minerals, (f), 1005 cm^{-1} representing overlap of carbohydrate, biogenic silica/silicate minerals. The symbols and sample dates indicate the midpoint of sample cup deployment periods. The gray line and squares represent EM; the black line and plus marks present WM.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Table 1. TOC, TN, molar C/N ratio, concentration of THAA, THAA–C/TOC, THAA–N/TN, and mol% composition of the 17 THAAs in the selected 10 sediment trap samples covering both sites (EM vs WM) and seasons (winter vs summer). The sampling date listed (month-day year) is the middle day of the 10-11 days for each collection period.

		EM					WM				
Sample date		1-14	4-12	5-26	7-31	8-14	10-20	1-11	4-12	5-26	7-26
		2010	2010	2010	2010	2010	2009	2010	2010	2010	2010
Amino acids* mol%	ASP	11.4	16.1	11.5	15.2	9.9	18.1	11.3	8.4	12.9	12.4
	GLU	8.2	12.4	8.9	10.4	7.5	13.4	8.1	7.2	9.7	9.6
	SER	9.7	6.8	8.1	9.5	8.1	3.3	10.1	8.2	8.5	9.1
	HIS	10.9	9.9	10.0	10.6	0.9	7.1	21.0	8.2	11.0	11.2
	THR/GLY	16.5	22.0	14.8	26.0	41.5	9.9	2.7	16.0	16.9	18.6
	ALA	17.5	5.0	16.4	6.0	3.1	17.8	19.7	15.6	14.4	19.8
	ARG	0.5	8.7	0.9	11.0	13.0	0.1	0.1	3.7	4.7	0.6
	TRY	0.8	1.1	0.9	1.2	3.1	2.9	0.5	0.5	0.1	0.4
	VAL/CYS	8.3	7.4	10.4	1.7	1.3	5.7	7.6	3.5	3.4	4.8
	MET	2.2	1.9	3.6	1.5	0.0	6.7	0.4	6.2	4.6	2.5
	PHE	0.4	0.6	3.1	0.7	0.8	0.1	4.6	1.3	0.8	0.1
	LLE	0.2	0.3	1.2	0.1	1.1	2.5	4.0	5.0	1.8	1.1
	LEU	3.3	2.0	1.9	1.6	1.9	3.1	1.2	4.3	2.2	1.9
	LYS/PRO	10.1	5.8	8.3	4.6	7.8	9.3	8.8	8.5	8.9	7.9
OC (%)		6.1	7.6	6.5	6.9	9.6	6.6	5.4	5.7	6.5	8.3
N (%)		0.7	0.9	0.7	0.8	1.3	0.9	0.7	0.7	0.7	1.0
molar C/N		10.0	9.7	10.8	10.0	9.0	8.9	9.5	9.3	10.6	9.8
THAA (mg/g)		21.1	16.8	12.9	26.9	39.7	18.6	20.3	22.5	28.5	50.4
THAA-C/TOC		14.6	9.1	8.5	15.9	17.0	12.0	16.6	17.1	18.4	25.0
THAA-N/TN		52.1	35.7	31.3	67.0	58.9	36.3	56.1	54.1	73.7	90.2

*L-aspartic acid (ASP), L(+)-glutamic acid (GLU), L-serine (SER), L-histidine (HIS), L-glycine (GLY), L-threonine (THR), L-arginine (ARG), L-alanine (ALA), L-tyrosine (TYR), L-cysteine (CYS), L-valine (VAL), L-methionine (MET), L-phenylalanine (PHE), L-isoleucine (LLE), L-leucine (LEU), L-lysine hydrochloride (LYS), L-proline (PRO).